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KLOTHO AND SMOKING - AN INTERPLAY INFLUENCING THE SKELETAL MUSCLE FUNCTION DEFICITS THAT OCCUR IN COPD

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ABBREVIATIONS LIST:
BMI - Body mass index
COPD - Chronic obstructive pulmonary disease
ELISA - Enzyme linked immunosorbent assay
FEV_1%pred - Forced expiratory volume in 1 second, expressed as a percentage of predicted value
FFM - Fat free mass
FFMI - Fat-free mass index
FGF-23 - Fibroblast growth factor-23
QMVC - Quadriceps maximal voluntary contraction

ABSTRACT

BACKGROUND:

Klotho is an 'anti-ageing' hormone and transmembrane protein; *Klotho* deficient mice develop a similar ageing phenotype to smokers including emphysema and muscle wasting. The objective of this study was to evaluate skeletal muscle and circulating Klotho protein in smokers and COPD patients and to relate Klotho levels to relevant skeletal muscle parameters. We sought to validate our findings by undertaking complimentary murine studies.

METHODS:

Fat free mass, quadriceps strength and spirometry were measured in 87 participants (61 COPD, 13 'healthy smokers' and 13 never smoking controls) in whom serum and quadriceps Klotho protein levels were also measured. Immunohistochemistry was performed to demonstrate the location of Klotho protein in human skeletal muscle and in mouse skeletal muscle in which regeneration was occurring following injury induced by electroporation. In a separate study, *gastrocnemius* Klotho protein was measured in mice exposed to 77 weeks of smoke or sham air.

RESULTS:

Quadriceps Klotho levels were lower in those currently smoking (p=0.01), irrespective of spirometry, but were not lower in patients with COPD. A regression analysis identified current smoking status as the only independent variable associated with human quadriceps Klotho levels, an observation supported by the finding that smoke exposed mice had lower *gastrocnemius* Klotho levels than sham exposed mice (p=0.005). Quadriceps Klotho levels related to local oxidative stress but were paradoxically higher in patients with established muscle wasting or weakness; the unexpected relationship with low fat free mass was the only independent association. Within locomotor muscle, Klotho localized to the plasma membrane and to centralized nuclei in humans and in mice with induced muscle damage. Serum Klotho had an independent association with quadriceps strength but did not relate to quadriceps Klotho levels or to spirometry.

CONCLUSIONS:

Klotho is expressed in skeletal muscle and levels are reduced by smoking. Despite this, quadriceps Klotho protein expression in those with established disease appears complex as levels were paradoxically elevated in COPD patients with established muscle wasting. Whilst serum Klotho levels

were not reduced in smokers or COPD patients and were not associated with quadriceps Klotho protein, they did relate to quadriceps strength.

INTRODUCTION

Klotho is an ageing suppressor gene, which encodes a single pass transmembrane protein, known to be expressed in various tissues including the kidney, parathyroid gland, choroid plexus and skeletal muscle. Circulating Klotho arises either directly from transcription of a truncated, secretable form of the protein or by cleavage of the extracellular domain of the full length transmembrane protein (1, 2). *Klotho* deficient mice develop an ageing phenotype characterised by a shortened life span and the clinical features of Chronic Obstructive Pulmonary disease (COPD) including emphysema and sarcopenia (3, 4). Consequently, reduced Klotho may contribute to some of the systemic aspects of COPD, including skeletal muscle function deficits that significantly impact morbidity and mortality (5, 6). As skeletal muscle retains plasticity in response to anabolic stimuli, treating this aspect of the disease is an attractive therapeutic option (7) making Klotho a candidate target in COPD.

Further evidence for a putative role of Klotho in the muscle dysfunction of COPD comes from a range of studies in man. Serial data from a single cohort of older adults in a general population, has linked low circulating Klotho levels with reduced grip strength (1), disability (8) and increased mortality (9). Data on Klotho in COPD had previously been restricted to two studies which indirectly investigated the effects of genetic polymorphisms which might influence Klotho expression (10, 11). Very recently, systemic Klotho levels and airway Klotho expression have been demonstrated to be lower in patients with COPD but the relevance to skeletal muscle function was not assessed (12, 13). Furthermore, current smoking status seems to attenuate circulating (14, 15) and airway (12, 13) Klotho levels. Since in developed countries smoking is the usual aetiological factor in COPD, a pathophysiological role for Klotho signalling in the development of skeletal muscle function deficits is possible; however there are currently no data available on human Klotho protein within skeletal muscle.

To assess a role for Klotho in the aetiology of skeletal muscle dysfunction in COPD, we addressed the following specific hypotheses. First, that Klotho protein can be detected in human skeletal muscle and that levels are reduced in the quadriceps of COPD patients and smokers. Second, that quadriceps Klotho levels are most reduced in patients with skeletal muscle function deficits and that local levels relate to local oxidative stress. Third, that serum and quadriceps Klotho levels would relate to phenotypic aspects of muscle function in COPD. To better understand the effects of Klotho within skeletal muscle we also evaluated Klotho expression in a smoking mouse model and assessed its expression in the context of muscle damage induced by electroporation. Because Klotho is a co-

receptor for Fibroblast growth factor-23 (FGF-23), where possible we also sought to evaluate FGF-23 levels.

METHODS

Human studies

Studies were conducted in accordance with the amended Declaration of Helsinki, ethics committee approval was obtained (West London REC 3: 10/H0706/9) and participants gave informed written consent. COPD was diagnosed according to GOLD guidelines (16). Exclusion criteria included being younger than 40 or older than 90 years of age, exacerbation or use of oral corticosteroids within the preceding 4 weeks, significant neurological or musculoskeletal limitation, inflammatory disease, respiratory disease (other than COPD) or cardiac, renal or hepatic disease. Never smokers (n=13), smokers with normal spirometry termed 'healthy smokers' (n=13) and COPD patients (including current smokers and former smokers) were recruited (n=61). Demographic data were recorded and spirometry (17), Body mass index (BMI), fat free mass (FFM) by bioimpedence (18) and dominant leg isometric quadriceps maximum voluntary contraction (QMVC) (19) were measured. Fat free mass index (FFMI) was calculated from the bioimpedance data (18). Patients were identified as having skeletal muscle wasting or quadriceps weakness by using recognised cut-offs (FFMI <15kg/m^2 in females or 16kg/m^2 in males and QMVC/BMI <1.20) (5, 6).

Percutaneous *vastus lateralis* biopsies were performed as previously described (20). Immunohistochemistry was performed to assess the localization of Klotho in muscle and to determine fibre proportions (21). Klotho (IBL, Japan), FGF-23 (EMD Millipore, MA, USA) and protein carbonyl levels (Biocell, Papatoetoe, NZ) were determined by enzyme linked immunosorbent assay (ELISA) from muscle protein extracts. Serum Klotho and FGF-23 levels were also determined by ELISA.

Mouse studies

The localization of Klotho was assessed in mouse muscle regenerating after injury caused by electroporation of a control DNA plasmid in experiments approved by the Royal Veterinary College Ethical Review Process (ERP-A-2010-WS01) and licensed by the UK Secretary of State for the Home

Office as Project Licence PPL 70/6797. The methodology for this is described elsewhere and is detailed further in the online supplement (22).

In a second and independent mouse study Klotho levels were determined from the *gastrocnemius* of female C57BL/6 mice exposed to either sham or smoked air inhalation over a 77 week period as described elsewhere (23).

Statistical analyses

Statistical analyses and graphical presentations were performed using GraphPad Prism 5 (GraphPad Software, San Diego, USA) or SPSS version 18 (IBM, USA). Significance was set at a 2-tailed p-value of ≤0.05. Parametric data are presented as mean (SD) and non-parametric data are presented as median (25^{th}, 75^{th} centiles).

A complete report of the Methods and a summary of the relevant studies undertaken is included in the online supplement, as is a more detailed description of the statistical analyses.

RESULTS

The characteristics of the 87 participants with serum and quadriceps Klotho measurements are shown in Table 1. The groups were well matched except that 'healthy smokers' were significantly younger than both never smokers and COPD patients; this is relevant since in population studies circulating Klotho falls with increasing age (8). The COPD patients had evidence of quadriceps weakness.

Human quadriceps Klotho expression

Quadriceps Klotho protein levels were not different between never smokers, 'healthy smokers' and COPD patients (Figure 1A). When combined irrespective of spirometric status, despite being a mean of 6 years younger, current smokers had lower quadriceps Klotho levels than former smokers (p=0.01; Figure 1B and e-Table 1). When confining the analysis to patients with COPD, current smokers still had reduced quadriceps Klotho levels (p=0.02; Figure 1C and e-Table 2).

Protein carbonyl determinations were available in 68 participants: 7 never smokers, 13 'healthy smokers' and 47 COPD patients, levels did not differ between these groups; ANOVA p=0.36, nor were they different in current smokers whether compared with either all non-current smokers (p=0.88) or never smokers (p=0.86). Across the cohort as a whole, protein carbonyls did not correlate with FFMI, QMVC, or QMVC/BMI, however, they did relate weakly to quadriceps Klotho protein levels (r=0.32, p=0.009), this relationship was also observed when limiting the analysis to COPD patients (r=0.34, p=0.02; e-Figure 1).

To identify the most relevant factors associated with quadriceps Klotho levels, regression analyses were performed. In the cohort as a whole (Table 2), only current smoking was observed to relate to quadriceps Klotho levels. When limiting the analysis to patients with COPD (e-Table 3) current smoking did not relate to quadriceps Klotho levels, but local protein carbonyls and the presence of a reduced FFMI did; the association with reduced FFMI was the only independent relationship observed.

Unexpectedly, we observed increased quadriceps Klotho levels in patients with a reduced FFMI (p=0.02; Figure 2A) and Klotho tended to be increased in patients with reduced QMVC/BMI (p=0.06; Figure 2B). Patients with both reduced FFMI and QMVC/BMI had higher quadriceps Klotho levels as

compared to those with preserved FFMI and QMVC/BMI (Kruskal Wallis p=0.008; Figure 2C). There was no difference between the proportions of current smokers and former smokers between each of these groups; p=0.40. Although a similar trend was observed between protein carbonylation and Klotho levels in patients with a reduced FFMI and reduced QMVC/BMI (e-Figure 2; e-Tables 4 and 5), only COPD patients with reduced QMVC/BMI and FFMI (n=6) were observed to have increased protein carbonylation and increased Klotho protein levels.

FGF-23 levels were very low and all but one determination was lower than the lowest standard concentration; subsequently FGF-23 levels were not evaluated any further.

Localisation of skeletal muscle Klotho

The presence of Klotho protein within human muscle was confirmed and Klotho was demonstrated to be localized to the muscle fibre membrane and to be associated with centralised nuclei (Figure 3A). Immunohistochemistry was available to provide fibre type data from *vastus lateralis* biopsies in 65 participants (9 never smokers, 13 'healthy smokers' and 43 COPD patients). There was no relationship between quadriceps Klotho levels and fibre type proportions either in the cohort as a whole or in COPD patients considered alone (r=0.04, p=0.95 and r=-0.20, p=0.21 respectively).

Animal Studies

Immunohistochemistry on the *gastrocnemius* muscles of electroporated mice confirmed significant Klotho expression to be present in damaged skeletal muscle tissue, but not healthy tissue, furthermore, Klotho co-localized to both the plasma membrane and to centralized nuclei (Figure 3B).

In mice exposed to smoke (n=19), there was a reduction in *gastrocnemius* Klotho levels as compared to those sham exposed (n=9); p=0.005, Figure 4.

Klotho in the circulation

There was no correlation between serum and quadriceps Klotho levels (r=0.17, p=0.13). Circulating Klotho levels and demographic data are shown in e-Figure 3 and Table 1. Serum Klotho levels did not differ between never smokers, 'healthy smokers' or COPD patients (e-Figure 3).

Klotho levels correlated with quadriceps strength, measured as QMVC/BMI, in the cohort as a whole ($r=0.37$, $p<0.0001$; e-Figure 4) and also when limiting the analysis to patients with COPD ($r=0.29$, $p=0.02$). To establish whether serum Klotho levels related to QMVC/BMI after accounting for other relevant variables, multiple regression analysis was performed. Serum Klotho maintained an independent relationship with QMVC/BMI (e-Table 6).

DISCUSSION

The main finding of this study is that quadriceps Klotho protein expression is lower in current smokers, whether evaluated in patients with COPD alone and or when also including individuals with normal spirometry. A causal role for cigarette smoking was confirmed by the use of a smoking mouse model. Within skeletal muscle, Klotho is located on the muscle fibre membrane and associated with centralised nuclei, suggesting Klotho may have a role in regeneration, consistent with the known sarcopenia demonstrated by Klotho deficient mice. Unexpectedly, the weakest COPD patients displayed the highest Klotho levels suggesting that Klotho mediated pathways are active, albeit that activity did not prevent muscle loss, and we demonstrated using electroporated mice that upregulation of Klotho can occur in the setting of muscle damage.

Critique of the method

Cross-sectional human studies are always vulnerable to the suggestion that observations may be epiphenomena rather than representing causality. Ultimately this dilemma can only be resolved by the use of an interventional study in man and we are not aware of any currently available Klotho mimetics with which this experiment could be tried. Nevertheless, we attempted to mitigate this risk both by studying a large number of human subjects and by undertaking supportive animal studies.

Klotho has, to our knowledge, never been specifically measured in human muscle before, therefore our findings whilst novel, also require confirmation, especially since in the case of FGF-23, for which it is a co-receptor, we found that levels were too low to be meaningfully interpreted. The finding of increased quadriceps Klotho levels in patients with established skeletal muscle wasting or weakness and of a positive, if weak, relationship with local oxidative stress were unexpected. It was hypothesised that elevated Klotho levels may be a physiological response; such a protective role was supported by a physical association with centralised nuclei, the hallmark of regenerating fibres. These findings were corroborated in regenerating mouse muscle where there was demonstrated to be significant Klotho expression and, as in humans, Klotho co-localized to centralised nuclei in regenerating fibres.

Significance of the findings

Historically the cachexia of COPD tended to be regarded as an end-stage event; however, several lines of data now support the concept of locomotor muscle atrophy and weakness as an early feature of the disease, supporting a connection with smoking which precedes clinical COPD. Firstly, lower limb muscle weakness is more prevalent than loss of whole body fat free mass (24, 25) in COPD. Secondly, it has been clearly demonstrated that *rectus femoris* cross sectional area and quadriceps maximal voluntary contraction force are reduced in patients with mild disease, in whom there is only minimal pulmonary impairment to exercise (26). Thirdly, muscle biopsy studies have shown changes which we would now recognise as typical of COPD, such as type II fibre shift or loss of oxidative enzymes, in smokers compared with non-smokers (27) and of oxidative stress in smokers with normal spirometry (28). Similarly, Gagnon *et al* (29) found evidence of impaired angiogenic signalling in the quadriceps of patients with minimal airflow obstruction, however, as controls had a very similar tobacco history to the patients it was impossible to evaluate the effect of smoking. Fourthly, muscle weakness was found to be common amongst breathless patients assessed in community practice even when spirometry was within the normal range (30).

If muscle weakness is accepted as an early feature of COPD, then the question arises as to the mechanism which causes it. Rightly, much attention has focused on physical activity which is indeed reduced in patients with mild disease (29, 31). Cigarette smoking is another candidate mechanism; Van den Borst and co-workers were able to show that body composition was very similar between patients with obstructive lung disease and smokers without obstructive lung disease and that both groups differed from never smokers, again supporting a prior triggering role for smoking (32). Similarly, Van Remoortel and co-workers (33) found co-morbidities including muscle weakness to be as prevalent in smokers with normal lung function as in patients with previously undiagnosed COPD, and in both cases to be more prevalent than in never smokers. More recently, it has been demonstrated that Klotho is reduced in the airway epithelial cells and alveolar macrophages of both smokers and COPD patients (12, 13) and that cigarette smoke reduces Klotho expression which subsequently confers augmented inflammation (13). Putative roles for smoking and physical activity in the aetiology of skeletal muscle weakness are not mutually exclusive. It would be insightful to know in the context of the present data whether Klotho genotype influences the response to exercise training or, alternatively, whether smoking status influences this response. Data on the latter are to our knowledge confined to one small scale study reported in abstract form (34).

The role of Klotho within skeletal muscle is not demonstrated by our findings, however based on data in the literature the most plausible proposal is that Klotho acts through the attenuation of

oxidative stress, which would be consistent with our finding of a relationship between quadriceps Klotho levels and protein carbonylation. Yamamoto *et al* showed that recombinant Klotho protected cells from oxidative stress *in vitro* and that manganese superoxide dismutase was increased in the muscle of *Klotho* over-expressing mice - consistent with a protective effect *in vivo*. However, they did not measure Klotho levels in muscle, indeed the mouse model used over-expressed *Klotho* in the brain and testis, suggesting that effects on muscle gene expression were mediated by circulating Klotho (35). Oxidative stress is increased in the quadriceps of smokers and COPD patients, in whom it relates to skeletal muscle dysfunction (36). Although we only demonstrated increased oxidative stress in weak patients, smokers with normal spirometry exhibit evidence of nitrosative stress (28). Recent work demonstrates an association between Klotho expression and smoking and that this interaction affects oxidative stress in the lungs. Li *et al* demonstrated that knockdown of endogenous Klotho augments the expression of inflammatory mediators, including MMP-9, IL-6 and TNF-alpha by alveolar macrophages and that exogenous Klotho inhibited the expression of cigarette smoke induced inflammation (13). This group extended their work to demonstrate Klotho depletion increases sensitivity to cigarette smoke-induced inflammation and oxidative stress-induced cell damage in epithelial cells where these effects involved the nuclear factor κB, mitogen-activated protein kinase and nuclear factor erythroid 2-related factor 2 pathways (12).

Modulation of Klotho or the effects of reduced Klotho offers a potential therapeutic opportunity in COPD; Nagai *et al* were able to show that an anti-oxidant, α-tocopherol, attenuated the manifestations of oxidative stress in the brain of *Klotho* knockout mice (37). While α-tocopherol was shown to be safe in over 29,000 smokers, measures which might have detected a beneficial effect on skeletal muscle were not obtained (38). Nonetheless, consistent with our own findings, a large prospective study has recently observed plasma Klotho levels to be higher in those with greater lower limb strength, furthermore baseline Klotho levels were predictive of loss of strength over time (39). In the present study, systemic and quadriceps Klotho levels were simultaneously measured for the first time and we observed that local and systemic levels were unrelated. This may be a consequence of Klotho being expressed in multiple tissues and in this regard circulating Klotho may reflect overall Klotho activity whilst local quadriceps Klotho protein levels may be influenced by a combination of local factors (for example contractile history) in addition to circulating levels. Despite this complex interplay, given that myotube diameter is increased by Klotho-FGF23 fusion polypeptide treatment, Klotho augmentation warrants further study, especially in smokers (36).

In conclusion, smokers irrespective of spirometric status, were found to have reduced quadriceps expression of Klotho, an effect replicated in a smoking mouse model suggesting a role for Klotho as a mediator of quadriceps weakness in COPD patients. However, in electroporated mouse experiments we show a role for Klotho in regeneration of damaged muscle. Since Klotho levels were also higher in the quadriceps of patients with muscle weakness we suspect that Klotho mediated regeneration may be attempting to restore skeletal muscle function, albeit with incomplete success.

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Conflicts of interest: none

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Author's contributions

MSP and MIP drafted the manuscript. MSP, AVD, SAN and DM recruited the patients and collected the physiological data and blood samples. MSP and AVD performed the *vastus lateralis* biopsies. BJB, JPF and PLP developed the smoked mouse model and implemented this over a 77 week period, providing the *gastrocnemius* muscles for further analysis. YA, SJa and PB performed immunohistochemistry on the human *vastus lateralis* samples with subsequent analytical input. All authors contributed to the analysis of data and preparation of the final manuscript, with intellectual input from SAN, NH, NSH, WD-CM and PK. PK supervised the molecular work that was performed by MSP, AL and JL. MSP and MIP conceived the idea; MIP is the primary investigator who takes responsibility for the integrity of the work as a whole, from inception to published article.

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TABLES

Table 1. Demographics and characteristic data of never smokers, 'healthy smokers' and COPD patients, groups differences are assessed by Chi-square and ANOVA or Kruskal-Wallis. *, ** or *** denote significant difference at p <0.05, <0.01 and <0.001 respectively as compared to never smokers and ǂ, ǂǂ or ǂǂǂ denote significant difference at p <0.05, <0.01 and <0.001 respectively as compared to 'healthy smokers'. Data expressed as mean ± SD or median (IQR).

	Never smokers (n=13)	'Healthy smokers' (n=13)	COPD (n=61)	P-value
Age (years)	65 (8)	51 (7) ***	64 (10)	**<0.0001**
% Male	77	38	62	0.12
Pack years	0 (0)	38 (28) ***	45 (24) ***	**<0.0001**
% Current smokers	0	100	46	**<0.0001**
FEV_1 (%pred)	108 (12)	99 (10)	51 (24) ***	**<0.0001**
BMI (kg/m^2)	26.4 (4)	28.2 (7)	26.5 (7)	0.66
FFMI (kg/m^2)	19.1 (4)	18.3 (3)	17.8 (3)	0.39
QMVC (kg)	37.9 (9)	40.1 (12)	30.5 (10) ǂ	**0.003**
QMVC/BMI	1.45 (0.3)	1.44 (0.4)	1.20 (0.4)	0.05

Abbreviations: BMI - Body mass index, FEV_1%pred - Forced expiratory volume in 1 second, expressed as a percentage of predicted value, FFMI - Fat-free mass index, QMVC - Quadriceps maximal voluntary contraction.

Table 2. Univariate regression analysis to determine the parameters associated with quadriceps Klotho levels (n=87).

	Variable	**Mean (SD)/ %**	**Univariate regression**		
			Coefficient (95% CI)	**Standardised coefficent**	**p**
Demographics & spirometry	Age (years)	62 (10)	0.14 (-0.07, 0.36)	0.15	0.17
	Male gender	66	1.8 (-2.7, 6.3)	0.09	0.43
	Current smoker	48	**-5.1 (-9.4, -0.9)**	**-0.25**	**0.02**
	COPD	70	0.03 (-4.8, 4.8)	0.01	0.99
	FEV_1 (%pred)	67 (32)	0.01 (-0.06, 0.08)	0.03	0.80
	BMI (kg/m^2)	26.7 (6)	-0.17 (-0.51, 0.17)	-0.11	0.32
Muscle parameters	FFMI (kg/m^2)	18.1 (3.1)	-0.22 (-0.92, 0.48)	-0.07	0.53
	QMVC (kg)	33.1 (11)	-0.14 (-0.34, 0.06)	-0.15	0.16
	QMVC/BMI	1.28 (0.42)	-2.2 (-7.4, 3.0)	-0.09	0.41

Abbreviations: BMI - Body mass index, FEV_1%pred - Forced expiratory volume in 1 second, expressed as a percentage of predicted value, FFMI - Fat-free mass index, QMVC - Quadriceps maximal voluntary contraction.

FIGURE LEGENDS

Figure 1 - Klotho levels in *vastus lateralis* muscle homogenates from **A)** Never smokers (n=13), 'healthy smokers' (n=13) and COPD patients (n=61), **B)** non-current smokers (never smokers and ex-smokers; n=46) and current smokers (n=41) irrespective of COPD status, and **C)** COPD former smokers (n=33) and COPD current smokers (n=28). Bars demonstrate the mean and standard deviation.

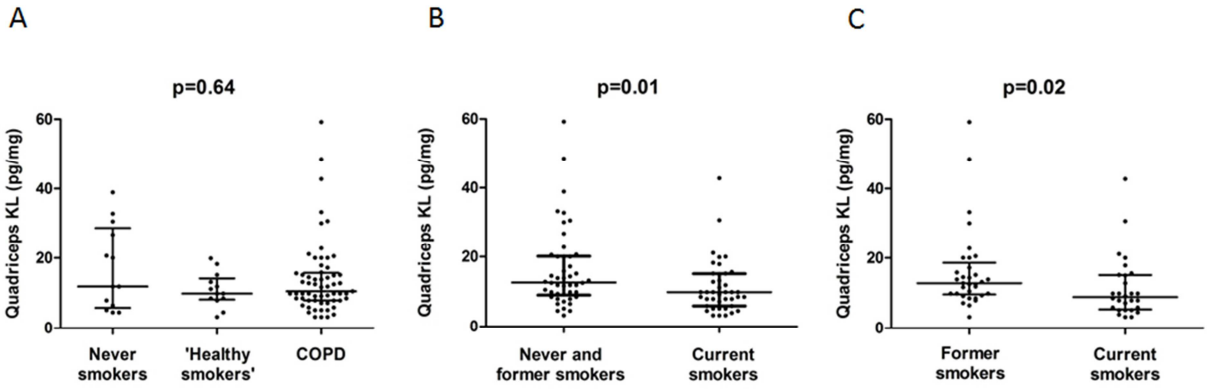


Figure 2 - Klotho levels in *vastus lateralis* muscle homogenates from COPD patients with **A)** preserved fat free mass (FFMI ≥15 in females or 16kg/m^2 in males; n= 45) as compared to those with reduced fat free mass (n= 16), **B)** preserved quadriceps strength (QMVC/BMI ≥1.20; n=30) as compared to those with loss of quadriceps strength (n=31), and **C)** preserved fat free mass and quadriceps strength (n=21) as compared to those with reduced quadriceps strength or fat free mass (n=32) and those with reduced quadriceps strength and fat free mass (n=8).

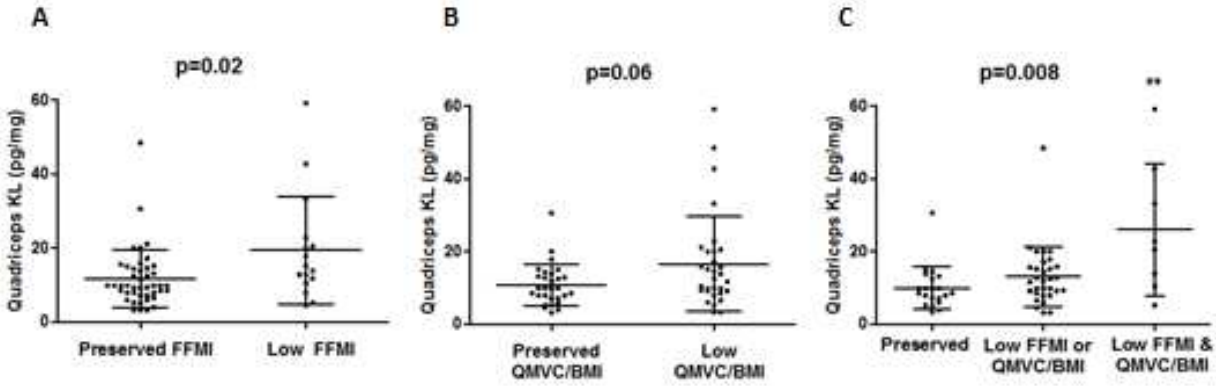


Figure 3 - Immunohistochemistry of **A)** human *vastus lateralis* muscle with blue DAPI nuclear staining only (left panel) and with Klotho staining green (centre panel) and a merged image with both Klotho and DAPI staining demonstrating Klotho to co-localize to an intracellular nucleus (right panel), and **B)** mouse *gastrocnemius* muscle with blue DAPI staining and Klotho staining in red, from a healthy mouse (left panel), at the site of injury following electroporation (centre panel) and distant to the site of injury following electroporation (right panel). Different colours are present for Klotho staining in human and mouse muscle due to the availability of secondary antibodies. The arrow highlights the presence of an intracellular nucleus in **A)** and in **B)** an example of one of numerous intracellular nuclei at the site of injured mouse muscle (centre), but only one distant to the site of injury (right).

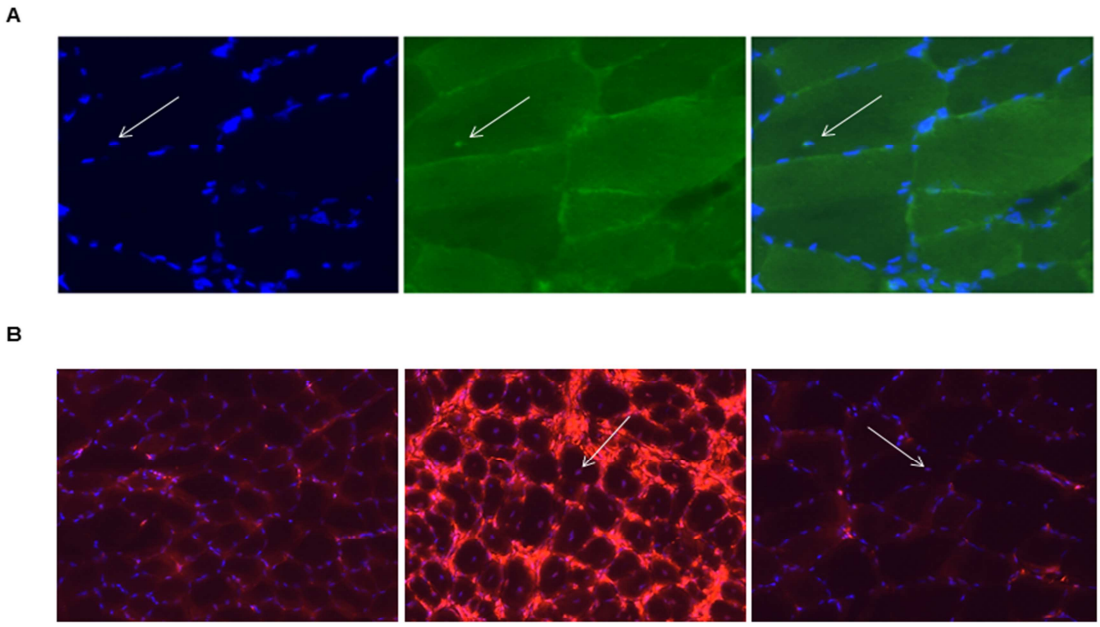
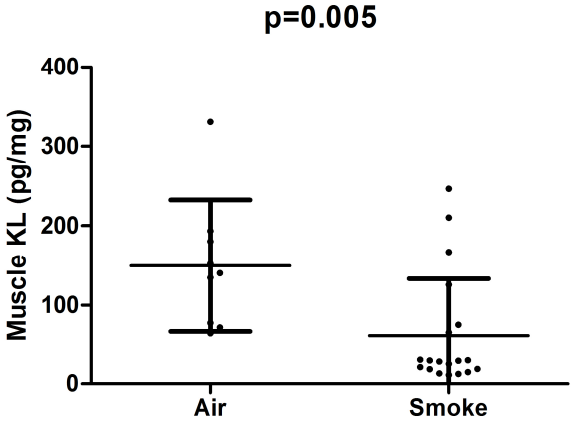


Figure 4 - Klotho levels in *gastrocnemius* muscle homogenates obtained from mice exposed to sham or smoked air over a 77 week period. Bars demonstrate the mean and standard deviation.



HIGHLIGHTS

- Klotho is expressed in skeletal muscle where levels are reduced by smoking
- In COPD, quadriceps Klotho levels are paradoxically raised in patients with wasting
- Klotho may have a role in the process of skeletal muscle regeneration
- Systemic Klotho levels are associated with quadriceps strength
- Smoking may mediate skeletal muscle dysfunction by influencing Klotho expression

Conflicts of interest: none

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Author's contributions

MSP and MIP drafted the manuscript. MSP, AVD, SAN and DM recruited the patients and collected the physiological data and blood samples. MSP and AVD performed the *vastus lateralis* biopsies. BJB, JPF and PLP developed the smoked mouse model and implemented this over a 77 week period, providing the *gastrocnemius* muscles for further analysis. YA, SJa and PB performed immunohistochemistry on the human *vastus lateralis* samples with subsequent analytical input. All authors contributed to the analysis of data and preparation of the final manuscript, with intellectual input from SAN, NH, NSH, WD-CM and PK. PK supervised the molecular work that was performed by MSP, AL and JL. MSP and MIP conceived the idea; MIP is the primary investigator who takes responsibility for the integrity of the work as a whole, from inception to published article.